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FUNGUS GALLS ON *CYSTOSEIRA* AND *HALIDRYS*

BY
LULA MAY ESTEE

W. G. FARLOW

UNIVERSITY OF CALIFORNIA PRESS
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INTRODUCTION.

Several species of *Cystoseira* are found along the Pacific Coast from southern California to Puget Sound. My attention was called by Professor W. A. Setchell to distortions found on one of these species, viz., on *Cystoseira Osmundacea,* and it was thought well to try to discover, if possible, the cause of these growths. Material was first collected at San Pedro by Miss Sarah P. Monks and later by Dr. N. L. Gardner. The material used in my study was collected by Dr. Gardner at San Pedro, California. It was in excellent condition and showed especially well the later stages of development. In most cases these growths were almost spherical in general outline and made up of numerous finger-like projections radiating from a center.

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[*] Originally presented as a thesis in partial fulfillment of the requirements for the degree of Master of Science in the College of Natural Sciences, University of California, May, 1912, but as published somewhat enlarged and changed.

Distortions of the same appearance and nature were also discovered by Dr. Gardner at San Pedro, but growing upon an undescribed species of *Halidrys,* distributed by Dr. Gardner under the name of *H. dioica* Gardner ms. (1911, Nos. CXVI and CXVII.)

HOST AND MATERIALS.

The brown alga, usually called *Cystoseira Osmundacea* (De Toni, 1895, p. 174), but referred to by Harvey (1858, p. 64) as *Halidrys Osmundacea* and by Ruprecht (1852, p. 12) as *Stephanocystis Osmundacea,* upon which these growths appear, grows attached by a disk-like root to rocks at about low-water mark. It reaches a length of five or six meters, is simply pinnatifid below with broadly linear leaves. A short distance from the base the plant becomes very much divided and in place of leaves there are spherical air vesicles arranged in series with deep constrictions between the vesicles. The number of vesicles on one branch-tip varies from one to eight. The average vesicles are about 3 mm. in diameter, becoming smaller towards the end of the series, those forming first and farthest from the tip of the branch being the largest. Beyond the ends of the air vesicles are the small fruiting receptacles.

The undescribed *Halidrys dioica* of Gardner (1911) is a plant similar in general habit and habitat to *Cystoseira Osmundacea.* One of the differences between the two species of interest here, is the fact that the seriate air vesicles are not deeply constricted from one another but form, as a series, a margined and siliquaeform structure (cf. plate 35, figs. 1 and 2).

CHARACTER OF DISTORTIONS.

The growths appear near the bases of the bladders (cf. plate 35, figs. 1 and 2), the smaller being nearer the tips of the branches, while the larger, older ones are farther back. They seldom grow on the solid, main thallus, but when found in such a position, they do not seem to develop normally but have almost globular rather than the finger-like projections such as are seen on the more healthy appearing distortions. These growths do not seem to attack the fruiting parts of the tips. They vary in size from those not more than 3 mm. in diameter and almost round to those 15 to 20 mm. in diameter and 10 mm. high. On the smaller distortions the projections are not more than 1 mm. long and 0.5 mm. in diameter, while those on the larger galls often reach 5 mm. in length and 1.5 mm. in diameter. There is no particular side of the thallus which these growths seem to favor, but where they do occur, the increased number of cells on the side infected produces a bend in the thallus. Their appearance is first noted as a tiny dark brown spot not more than 0.5 mm. in diameter. These little spots soon become wart-like, then lengthen into the finger-like projections with slightly enlarged tips.

TECHNIQUE.

The infected plants were sent to Berkeley immediately after being collected, and to preserve them for future study, they were placed in a 2 per cent. solution of formalin. After making a superficial examination of the distortions, I first made sections with a razor, and from these could obtain a good general idea of the enlargement of the thallus at the point infected and of the branching effect of the finger-like projections from this point. A small, circular, white spot could also be seen, which occupied about half of the width of the projection near its tip. Longitudinal sections of the finger-like projections were then made, using the freezing microtome, and these were very thin and smoothly cut. A number of different stains were experimented with in order to try to discover one which would differentiate the small white spot of foreign material from the algal cells surrounding it. The best results were obtained when using a very strong aqueous solution of Hanstein's aniline violet, staining the sections deeply and then partly decolorizing them by using dilute HCl. By removing the sections from the HCl and rinsing in water at just the proper moment, a beautiful differentiation of color was obtained, the cells of the alga being stained green and those of the fungus pink. The sections were then ready to dehydrate with alcohol and mount, using either glycerine jelly or balsam, depending upon whether temporary or permanent mounts were desired.

HISTOLOGY.

Studying the longitudinal sections, two sorts of bodies, quite different in structure were found. First, the perithecia of some ascomycetous fungus (cf. plate 35, fig. 3), almost circular, from 522 to 922μ in diameter, with an opening from the perithecium at the tip of the finger-like projection. Five or six layers of unpigmented pseudoparenchymatous cells make up the outer wall of the perithecium. Within this is a thin layer of less deeply stained tissue from which club-shaped asci arise. These, in varying stages of maturity, interspersed with empty asci line the sides and base of the perithecium, while the portion just below the opening and the opening itself are lined with short hyphal branches, directed slightly upward towards the opening. These branches almost meet, very nearly closing the opening. The asci (cf. plate 35, figs. 4 and 5) are from 90 to 145μ long and from 9 to 30μ wide. Each ascus contains eight spores which arrange themselves in two rows, and when mature seem to be set free in the interior cavity, filled with a jelly-like mass, by the dissolving of the walls of the ascus, and when the pressure is sufficiently great, they are forced out with this jelly through the opening of the perithecium. The spores (cf. plate 35, fig. 6) are ellipsoidal, about three times as long as broad, and when mature have a granular band across the center. The spores have a vertical diameter of from 30 to 45μ and horizontal diameter of from 9 to 13μ.

In some of the more nearly spherical rather than finger-like projections there are egg-shaped bodies, with long diameter of from 444 to 622μ and short diameter from 277 to 444μ, somewhat smaller than the perithecia, with pseudoparenchymatous walls of hyphae, finer and more delicate than those in the perithecia. These bodies seem to be of the nature of spermagonia (cf. plate 35, fig. 7).

From the wall as seen in longitudinal section, pseudoparenchymatous cells extend towards the center of the spherical cavity in the form of five to eight low, blunt triangles, which vary considerably in size and are irregularly placed along the wall. Short, slender, unbranched hyphae project from these triangular masses and from the wall between their bases, thus lining the entire central cavity. With the exception of the hyphae lining the opening and immediate vicinity, they abjoint successively small, oval spores (plate 35, fig. 8) from their tips, about 3μ long and 1.5μ wide, which remain in strings extending almost to the center of the interior cavity. The spores lie in a jelly-like substance with which the central cavity is filled, until the pressure becomes great enough to force the jelly containing them through the opening, which lies usually near the tip of the finger-like projection. The pore, in the case of the spermagonium, is inverted funnel-shaped rather than cylindrical as in the perithecium.

In the perithecium there are pseudoparenchymatous cells along the base of the tubelike opening which project inward toward the center of the cavity, making the tubelike opening extend downward about one-third of the diameter of the circular, central cavity. No similar mass of cells is found in the spermagonium, there being a gradual slope of the wall from the center to the external opening.

The perithecia and spermagonia do not, so far as noted, occur in the same finger-like projection or in the same distortion. Judging from the fact that these spermagonia are found in projections similar to those containing perithecia, also from their likeness in form and structure, it is extremely probable that they are spermagonia and perithecia of the same species.

There is an increased number of cells in the wall of the perithecium very nearly opposite the opening, and from this mass of cells a strand of hyphae extends downward into the finger-like process of the gall. It would appear that the infection begins with this strand which grows in length, keeping pace with the growth of the finger-like projection. The strand gives off smaller strands at its sides and finally ends as a mass of hyphal strands, from which the perithecium develops. There are other strands of hyphae extending from the perithecium besides the basal strand, but they are shorter, finer and less branching. Much the same condition exists in the spermagonium except that the strands are more delicate, and if there are any extending from the sides of the spermagonium, they are so small that they are not readily seen.

IDENTIFICATION OF FUNGUS.

The fungus described above has a perithecium, globular or nearly so, entirely immersed in the host, through which it opens by a distinct pore. Asci line the interior of the perithecium but are not accompanied by paraphyses. The spores are hyaline, ellipsoidal, one-celled with a granular band across the center, when mature. These characters definitely indicate it as a species of *Guignardia* (ref. Engler & Prantl., *Die natürl. Pflanzenfam.*. vol. 1:1, p. 387).

It seems to be an undescribed species of *Guignardia*. No such fungus has been described as occurring on any of the *Fucaceae*. It may be compared with *Guignardia Ulvae* Reed which occurs on marine algae. The perithecium has a greater outside diameter than that of *G. Ulvae* and the asci and spores are considerably larger. The long, narrow spores also have pointed rather than rounded ends. The perithecium in our plant is from 522–922μ transverse outside diameter, the asci are 90–145μ by 9–30μ, and the spores are 9–13μ by 30–45μ, while *G. Ulvae* Reed has a perithecium which measures 364–509μ outside diameter and 120–270μ internal diameter. The asci are 23–40μ by 10–13μ and the spores measure 3.5–7μ by 10–13μ. *G. alaskana* Reed is described as having a perithecium whose external diameter varies from 273–450μ and the internal diameter from 110–216μ. The asci are club-shaped bodies 7–14μ by 23–40μ, and the spores 3–4μ by 8.5–13.5μ.

In searching through the literature on the subject, no species of *Guignardia* has been found having spore measurement coinciding with those of the parasite on *Cystoseira* and *Halidrys* nor occurring on the same or related hosts; furthermore no known species of this genus causes any such considerable distortion of its host; therefore, in connection with Professor W. A. Setchell, I propose the name *Guignardia irritans* for this new species, naming it from the galls which it produces.

Guignardia irritans Setchell et Estee sp. nov.

G. peritheciis immersis, sphericis, 522–922μ diam., poro apicali cylindicoque apertis, parietibus stratibus paucis pseudoparenchymaticis non pigmentatis; ascis clavatis, octosporis, 90–145μ longis, 9–30μ latis, parietes peritheciorum poro excepto totaliter vestientibus maturitate evanescentibusque; sporis hyalinis, oblongo-ellipticis, utrinque acute apiculatis, 30–45μ longis, 9–13μ latis, maturitate unicellularibus, zona granulata meridionali instructis; spermagoniis adparentur peritheciis late remotis, oblongo-ovatis, 444–622μ longis, 277–444μ latis, poro apicali cylindricoque instructis, parietibus iis peritheciorum valde similibus et intus sterigmatibus simplicibus completo vestitis; spermatiis hyalinis, late ellipticis, 3μ longis, 1.5μ latis, a sterigmatibus seriatim abstrictis et in gelina hyalina obvolutis. In frondibus prope vesiculas aeriferas seriatas *Cystoseirae Osmundaceae* et *Halidryos dioicae* parasitica et protuberantias gallaeformes, usque 20 mm. diam., processibus digitiformibus e centro solidiore radiantibus compositas efficiens.

Prope "San Pedro" in ditione Californiensi ubi detexerunt Domina Sarah P. Monks et Doctor N. L. Gardner.

FUNGI OCCURRING ON THE LARGER BROWN ALGAE.

As has been said above, no fungus parasites have been described as growing upon any of the *Fucaceae*. Farlow (1879, p. 10) mentions a species of "*Sphaeria*" which grows submerged in salt water, as a parasite on the stipes of *Laminaria longicruris*. another of the brown algae. Upon examination this proves to be very different from the fungus on *Cystoseira* and *Halidrys,* as its spores are distinctly two-celled. The fungus on *Laminaria longicruris* has been described as *Dothidella Laminariae* Rostr. (cf. Saccardo, 1895, p. 375). Upon only slightly modified portions of the thallus of *Halidrys dioica* (collected by Gardner) have also been found spermagonia which are very much like those occurring in the distortions, except that they are less in diameter, the hyphae which form the walls are more slender and the spores smaller. This second parasitic fungus on *Halidrys dioica* grows on the stems and is entirely submerged. *Cystoseira* and *Halidrys* live at or just below extreme low-water mark, but the position of the fungus galls near the air bladders may indicate that they were partially emergent.

NATURE OF PARASITISM.

Among distortions occurring on brown algae are those upon *Ascophyllum* and *Desmarestia* described by Ethel S. Barton (1892). She says that in the case of *Ascophyllum* they are confined to that part of the thallus immediately above or below the air vesicles. The portion affected is much enlarged and covered with round swellings or lumps. Each one of these swellings upon being cross-sectioned is found to be full of nematode worms. Miss Barton states that the swellings are probably the result of stimulation from some animal secretion. The distortions upon *Desmarestia* are similar to those found upon *Ascophyllum* but have thinner walls and smaller cells. Even though these distortions somewhat resemble those on *Cystoseira* they originate from an entirely different cause.

R. Valiante (1883, p. 489) tells of a parasitic plant collected by him in the Gulf of Naples, on *Cystoseira Opuntioides,* which he calls *Streblonemopsis irritans.* He was unable to find the germinating zoospore, but began his study with the incipient mammalate form. The small plants send out branches under the epidermis of the *Cystoseira,* forming small whitish spots about 1 mm. in diameter and sometimes as excrescences resembling little galls. The peripheral cells of the *Cystoseira* enlarge with the growth of the small white spots and the contents become, little by little, granular, but almost transparent. The greatest diameter reached by the protuberances was about 2 mm. Growth of the *Streblonemopsis irritans* takes place from an apical cell extending as a prolonged thread just under the epidermis and producing, at the same time, lateral swellings which prolong horizontally and form branches. Opposing branches come together, their apices unite and form meshes of the network. As a result of this manner of ramification, branches having the same base send out side branches and unite. Sauvageau (1892, p. 351) writes of his examination of *Streblonemopsis irritans* and says that it is found only on the numerous little tubercles on the *Cystoseira* which are almost sessile on the stem. The protuberances, he says, resemble little galls, beginning as one protuberance indicated by a small whitish patch, the enameled appearance of the epidermis returning with the recovery of the cortical tissue. At the surface of the protuberances are ramifications of the filaments which form a more or less abundant network of meshes under the epidermis. He also, by referring to the figures of Valiante, calls attention to the resemblance of the sporanges and filaments to the epidermis of the host. The galls described by these writers, although resembling the galls of our plant, are caused by a parasitic alga of the group of the *Phaeophyceae* and not by a fungus.

Sauvageau has described a distortion (Sauvageau, 1892, pp. 20 and 41) produced by still another parasitic brown alga and occurring upon *Cystoseira* collected in the Gulf of Naples, to which Bornet gives the name *Ectocarpus Valiantei.* In this case the galls are not scattered regularly over one surface but are very unequally distributed, sometimes in close bunches. When the parasitic germ on the surface of the *Cystoscira* penetrates the cells of the epidermis, it does not penetrate more than two or three layers of cells deep. In a short time there is a multiplication of the cells of the host invaded, in much the same manner as occurs commonly in galls caused by the sting of insects. The filaments of the parasite do not, therefore, invade the host to any extent but surround the multiplying mass of cells. In both these algal parasites, the growth is much more superficial than it is in the fungus parasite described in this paper, which sends a hyphal strand down a considerable distance among the tissues of the host.

OTHER FUNGI ON MARINE ALGAE.

By far the greater number of fungus parasites on algae are found upon fresh-water forms, and belong, chiefly, to the groups *Chytridineae* and *Ancylistineae.* These two groups are normally more or less aquatic and show certain resemblances to the *Saprolegniineae.* A fairly complete list of fungus parasites on algae is found in Lemmermann (1901, pp. 185–202). Of the one hundred and ninety-three species noted by Lemmerman, only ten are of the group *Pyrenomycetineae,* the group to which our fungus belongs, and only one of these, *Dothidella Laminariae* is found parasitic on marine algae. Reference has already been made in this paper to this species. Only three other species of the *Pyrenomycetineae,* parasitic on marine algae, besides those enumerated in Lemmermann's list and by Reed (1902) are known to me; one, *Zignoella calospora* (Patouillard, 1897, p. 242), parasitic upon *Castagnea chordariaeformis,* collected by Sauvageau at Gigon, Spain, has the perithecia superficial, paraphyses indistinct, spores hyaline, transversely septate three to six times; the second, *Zignoella enormis* (Patouillard et Hariot, 1903, p. 228) on *Stypocaulon scoparium,* with perithecia superficial and hyaline spores four or five times transversely septate. The descriptions of these two show them to be entirely different from the fungus parasite on *Cystoseira* and *Halidrys.* The third species, however, is much more closely related to the parasite on *Cystoseira* and *Halidrys.* This is *Sphaerella Chondri* of Herbert L. Jones (1898, p. 3). In all probability this fungus, found on *Chondrus crispus,* a marine red alga, at Nahant, Mass., belongs to the genus here called *Guignardia.* Besides being on a different host and not causing any considerable distortion, it is described as having more slender asci and decidedly more slender spores. It seems, therefore, to be clearly distinct from *Guignardia irritans.*

In conclusion, I wish to express my appreciation to Professor W. A. Setchell, with whom the work has been carried on, for his kindly suggestions and criticisms; also, to Dr. N. L. Gardner of the Polytechnic High School, Los Angeles, California, for furnishing the material used.

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EXPLANATION OF PLATE 35.

Fig. 1.—Part of a series of air vesicles of *Halidrys dioica*, showing a younger gall. × 2 diam.

Fig. 2.—Small section of *Halidrys dioica* with parts of three series of air vesicles, showing a mature gall on the thallus near the base of the air bladders. × 2 diam.

Fig. 3.—Median longitudinal section through the tip of one of the finger-like projections of a gall, showing a perithecium and the strand of branching hyphae descending (as a darker line) from its base, between the cells of the host. The larger asci are very nearly mature and have within them large ascospores, while the asci containing smaller spores are immature. In some of the asci the spores are rounded bodies, not having yet taken the form of the mature spores. The shaded portion between the asci is gelatinous, while the lines and blank spaces in the central cavity seem to be portions of mature asci which have been forced away from the wall of the perithecium. × 68 diam.

Fig. 4.—Young ascus with immature, unbanded ascospores. × 276 diam.

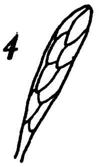
Fig. 5.—Mature ascus with mature spores, showing the central granular band. × 177 diam.

Fig. 6.—Mature spores, showing rather thick, gelatinous walls, much thicker at the ends than at the sides, and the granular band across the center. × 329 diam.

Fig. 7.—Longitudinal median section through a spermagonium, showing the variation in the shape of the cells of the spermagonium, from its wall in towards the spores, which seem to be given off, in strings, from the ends of the sterigmata. × 66 diam.; somewhat diagrammatic.

Fig. 8.—Very small, oval, hyaline spermatia. × 1400 diam.

All the drawings made by Lula May Estee with the aid of the Abbe camera lucida.



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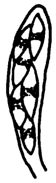

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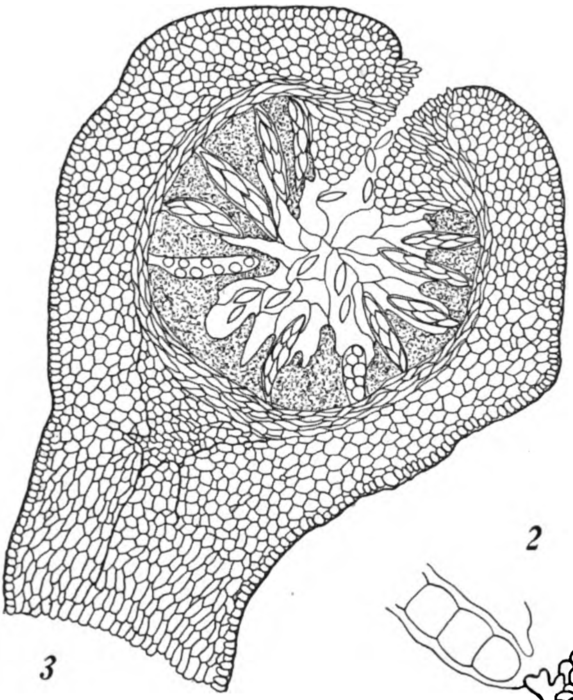

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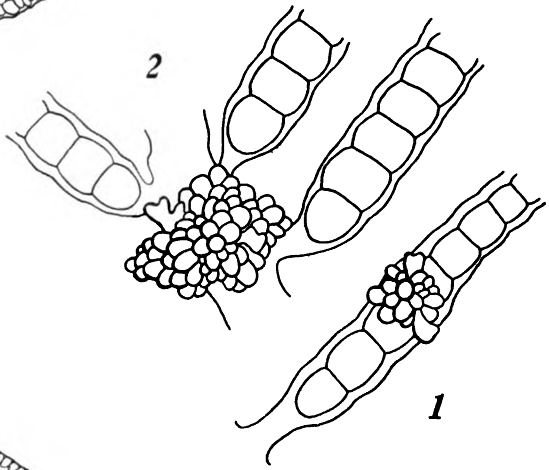

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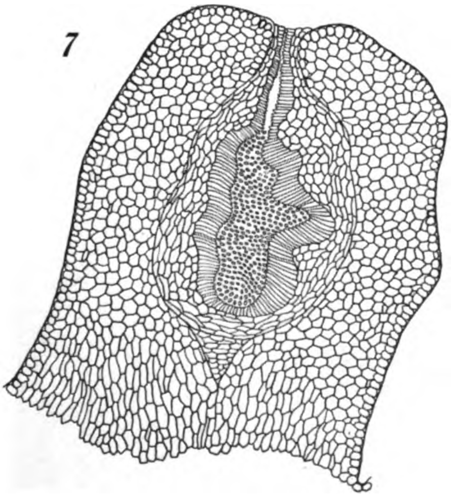

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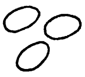

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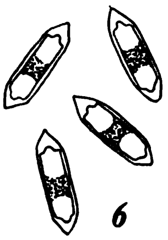

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